EIC SEARCH RESULTS

Serial No. 09/872,526 – Fetal tissue harvesting and implantation method

Searcher: Ethel Leslie
Date: August 6, 2008

Foreign & International Patent Search

Search Strategy

```
Set     Items   Description
S1      29558   S FETUS?? OR FOETUS?? OR FAETUS?? OR FETAL? OR FOETAL? OR
FAETAL? OR EMBRYO? ? OR EMBRYONIC?
S2        256   S (UNBORN? OR (UN OR "NOT" OR PRIOR OR BEFORE)(2W)(BORN??? OR
BIRTH???) OR PRE()(TERM? ? OR NATAL???) OR PRETERM? OR PRENATAL? OR UTERO OR
VIVO OR VENTER OR UTERUS? OR WOMB? ? OR UTERI? ? OR INUTERO? ?)(5N)(BABY? ?
OR BABIES OR CHILD? ? OR CHILDREN?)
S3      29759   S S1:S2
S4        281   S (MAINTAIN? OR SUSTAIN? OR REMAIN? OR CONTINU? OR STAY???)
(5N) (VIABLE? OR VIABILIT? OR LIFE OR ALIVE OR LIVE OR LIVES OR LIVED OR
LIVING OR SURVIV?)
S5      22872   S TISSUE? ? OR BONE OR MARROW? OR SPECIMEN? OR SAMPLE? ? OR
BLOOD OR OSTEOBLAST? OR CELLS OR STEMCELL? OR ORGAN? ? OR HEART OR HEARTS OR
LUNG OR LUNGS OR LIVER OR KIDNEY? OR GLAND? ?
S6      10290   S S5(5N)(EXTRACT? OR REMOV? OR HARVEST? OR BIOPSY? OR
BIOPSIE? ? OR SAMPLING? OR SAMPLE? ? OR RESECT? OR WITHDRAW? OR COLLECT? OR
EXCIS??? OR EXCISION? OR OBTAIN??? OR (TAKE? ? OR TAKING OR TOOK)()OUT)
S7        125   S S5(5N)(REINSERT? OR REINJECT? OR READMINISTER? OR RETURN?
OR REINTRODUC? OR REINDUCT? OR (PUT OR PUTS OR PUTTING)()BACK)
S8       4093   S S5(5N)(INSERT? OR INJECT? OR ADMINISTER? OR INTRODUC? OR
INDUCT?)
S9          7   S S3 (10N) (AUTOGEN? OR AUTOGRAFT? OR AUTO()GRAFT???)
S10        44   S S3 AND S4 AND S6 AND S7:S8
S11        44   S S10 NOT S9
S12        19   S S3 (2S) S4 (2S) S7:S8
S13        54   S S3(2S)S6(2S)S7
S14        62   S S12:S13 NOT (S9 OR S11)
S15      2377   S S5(5N)(IMPLANT? OR TRANSPLANT?)
S16        46   S S3 AND S4 AND S6 AND S15
S17        29   S S16 NOT (S9 OR S11 OR S14)

[File 350] Derwent WPIX 1963-2008/UD=200848
(c) 2008 The Thomson Corporation. All rights reserved.
[File 347] JAPIO Dec 1976-2007/Dec(Updated 080328)
(c) 2008 JPO & JAPIO. All rights reserved.
```

Search Results

No relevant results.

NPL Database Search

Search Strategy

```
Set     Items   Description
S1    1924332   S FETUS?? OR FOETUS?? OR FAETUS?? OR FETAL? OR FOETAL? OR
FAETAL? OR EMBRYO? ? OR EMBRYONIC?
S2      23679   S (UNBORN? OR (UN OR "NOT" OR PRIOR OR BEFORE)(2W)(BORN??? OR
BIRTH???) OR PRE()(TERM? ? OR NATAL???) OR PRETERM? OR PRENATAL? OR UTERO OR
VIVO OR VENTER OR UTERUS? OR WOMB? ? OR UTERI? ? OR INUTERO? ?)(5N)(BABY? ?
OR BABIES OR CHILD? ? OR CHILDREN?)
S3    1941743   S S1:S2
S4       8102   S (MAINTAIN? OR SUSTAIN? OR REMAIN? OR CONTINU?) (5N)
(VIABLE? OR VIABILIT? OR LIFE OR ALIVE OR LIVE OR LIVES OR LIVED OR LIVING OR
SURVIV?)
S5    1218144   S TISSUE? ? OR BONE OR MARROW? OR SPECIMEN? OR SAMPLE? ? OR
BLOOD OR OSTEOBLAST? OR CELLS OR STEMCELL? OR ORGAN? ? OR HEART OR HEARTS OR
LUNG OR LUNGS OR LIVER OR KIDNEY? OR GLAND? ?
S6     139109   S S5(5N)(EXTRACT? OR REMOV? OR HARVEST? OR BIOPSY? OR
BIOPSIE? ? OR SAMPLING? OR SAMPLE? ? OR RESECT? OR WITHDRAW? OR COLLECT? OR
EXCIS??? OR EXCISION? OR OBTAIN??? OR (TAKE? ? OR TAKING OR TOOK)()OUT)
S7       6859   S S5(5N)(REINSERT? OR REINJECT? OR READMINISTER? OR RETURN?
OR REPLAC? OR REINTRODUC? OR REINDUCT? OR (PUT OR PUTS OR PUTTING)()BACK)
S8      35779   S S5(5N)(INSERT? OR INJECT? OR ADMINISTER? OR INTRODUC? OR
INDUCT?)
S9        840   S S3(S)S6(S)S7
S10    273064   S PY=1991:1995
S11    347268   S PY=1996:2000
S12    310881   S PY=2001:2004
S13    288807   S PY=2005:2009
S14       226   S S9 NOT S10:S13
S15       140   RD  (unique items)
S16       147   S STAY???(5N) (VIABLE? OR VIABILIT? OR LIFE OR ALIVE OR LIVE
OR LIVES OR LIVED OR LIVING OR SURVIV?)
S17        62   S (S4 OR S16) AND S6 AND S7:S8
S18        10   S S17 NOT (S10:S13 OR S14)
S19         8   RD  (unique items)
S20        15   S AUTOGENIC?(5N)S5
S21         7   RD  (unique items)
S22       562   S AUTOGRAFT? OR AUTO()GRAFT???
S23        12   S S3 AND S22 AND S4
S24        96   S AUTOGEN?(5N)S5
S25         0   S S3 AND S24 AND S4
S26         0   S S23 NOT (S10:S13 OR S14 OR S18)
S27       152   S S3 AND S22 AND S6:S8
S28        15   S S27 NOT (S10:S13 OR S14 OR S18)
S29        12   RD  (unique items)
S30       123   S S3(10N)S22
S31        32   S S30 NOT (S10:S13 OR S14 OR S18 OR S28)
S32        22   RD  (unique items)
S33   1384722   S S3/TI,DE
S34       236   S S22/TI,DE
S35       152   S S33 AND S34
S36        15   S S35 NOT (S10:S13 OR S14 OR S18 OR S28 OR S31)
S37        15   RD  (unique items)
```

Search Results

15/7/9 (Item 9 from file: 155) **Links**
Fulltext available through: STIC Full Text Retrieval Options
MEDLINE(R)

09157407 **PMID:** 2814704
[Xipho-omphalopagus--Siamese twins with multiple abnormalities of the gastrointestinal tract]

Xiphoomphalophagus--siamske dvojcata s mnohopocetnymi malformaciami GIT.
Janec M; Vojtko M; Kubikova E
Rozhledy v chirurgii - m si nik eskoslovenske chirurgicke spole nosti (
CZECHOSLOVAKIA) Sep 1989 , 68 (8-9) p616-9 , **ISSN:** 0035-9351--Print
Journal Code: 9815441
Publishing Model Print
Document type: Case Reports; English Abstract; Journal Article
Languages: SLOVAK
Main Citation Owner: NLM
Record type: MEDLINE; Completed
The authors describe a case of xiphoomphalpagus; one infant was dead, the other alive. They had a common umbilical cord and omphalocele, joined liver, only one gallbladder and common duodenum in the shape of a wide sac. From this originated two thin guts, one was wide and belonged to the dead foetus, the gut of the live foetus beneath the duodenum was atretic. Already six

hours after delivery the authors separated the infants and in the live infant they not only **resected** the **liver** and **reinserted** the choledochus but also repaired the wide duodenum and atresia. The second infant died on the 4th day after operation from congenital heart disease incompatible with life. The stillborn infant has, as revealed by necropsy and histological examination, a congenital megacolon. The authors analyze the scope of diagnostic and surgical possibilities.
Record Date Created: 19891213
Record Date Completed: 19891213

15/7/12 (Item 12 from file: 155) **Links**
Fulltext available through: STIC Full Text Retrieval Options
MEDLINE(R)

08929954 **PMID:** 2920208

[redacted]

Kantoff P W; Flake A W; Eglitis M A; Scharf S; Bond S; Gilboa E; Erlich H; Harrison M R; Zanjani E D; Anderson W F
Laboratory of Molecular Hematology, National Heart, Lung, and Blood Institute, Bethesda, MD 20892.
Blood (UNITED STATES) Mar 1989 , 73 (4) p1066-73 , **ISSN:** 0006-4971--Print **Journal Code:** 7603509
Contract/Grant No.: AM2047; AM; United States NIADDK; HL40722; HL; United States NHLBI
Publishing Model Print
Document type: Journal Article; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.; Research Support, U.S. Gov't, P.H.S.
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Retroviral-mediated gene transfer was used to insert a Neo R gene into fetal sheep hematopoietic cells obtained by exchange transfusion from lambs in utero. After gene transfer the **cells** were **returned** to the donor **fetus**. The lambs were examined after birth for the presence of a functioning Neo R gene. Of ten analyzable animals, six were positive for G418 resistant progenitor cells (CFU-Mix, CFU-C, BFU-E, CFU-E). Two animals were studied for extended periods of time: 8 and 24 months. Each has demonstrated a pattern wherein positive periods are interspersed with times when there were no detectable G418-resistant cells. We conclude that retroviral-mediated gene transfer can be used to insert genes into early progenitor cells of **fetal** sheep in utero and that the animals can continue to demonstrate blood cells expressing the gene for more than 2 years after birth. This is a US government work. There are no restrictions on its use.
Record Date Created: 19890420
Record Date Completed: 19890420

15/7/24 (Item 24 from file: 155) **Links**
Fulltext available through: STIC Full Text Retrieval Options
MEDLINE(R)

08059311 **PMID:** 3766728
Continuous ovine fetal hemorrhage: sensitivity of plasma and urine arginine vasopressin response.

Ross M G; Ervin M G; Leake R D; Humme J A; Fisher D A
American journal of physiology (UNITED STATES) Oct 1986 , 251 (4 Pt 1)

pE464-9 , **ISSN:** 0002-9513--Print **Journal Code:** 0370511
Contract/Grant No.: HD-06335; HD; United States NICHD; RR-00425-1652; RR; United States NCRR
Publishing Model Print
Document type: Journal Article; Research Support, U.S. Gov't, P.H.S.
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Intravascular hemorrhage of the ovine fetus is a potent stimulus for arginine vasopressin (AVP) secretion. However, the method (acute, continuous) and rate of blood withdrawal may influence the fetal response. To determine the hemorrhage threshold for AVP secretion in response to slow continuous hemorrhage, five chronically catheterized ovine fetuses were continuously hemorrhaged (0.6% blood vol/min) to 24-30% blood volume withdrawal. Immediately after hemorrhage fetal blood was reinfused at an equivalent rate. In addition to AVP measurements by radioimmunoassay, fetal urinary responses were monitored as an index of fetal AVP secretion. Significant increases in plasma AVP occurred during hemorrhage (1.0 +/- 0.1 to 8.0 +/- 2.0 pg/ml). The fetal plasma AVP-hemorrhage threshold, as defined by regression analysis, occurred at withdrawal of 13.0% blood volume. Fetal urine volume significantly decreased from a mean basal rate of 0.59 +/- 0.03 to 0.21 +/- 0.06 ml/min at the completion of hemorrhage. Urinary sodium, potassium, and osmolar excretion also significantly decreased at the completion of hemorrhage. Urinary AVP excretion, urine osmolality, sodium, and potassium concentrations did not change significantly during the hemorrhage period but increased significantly during the reinfusion period; the delay a result of renal and catheter dead space. Reinfusion of **blood** resulted in a **return** of plasma AVP to basal levels. These results define a threshold for AVP secretion and demonstrate significant urinary effects in response to slow continuous hemorrhage.
Record Date Created: 19861110
Record Date Completed: 19861110

15/7/53 (Item 53 from file: 155) **Links**
Fulltext available through: STIC Full Text Retrieval Options
MEDLINE(R)

06091873 **PMID:** 45273
Plasma vasopressin levels during haemorrhage in mature and immature fetal sheep.

Rurak D W
Journal of developmental physiology (ENGLAND) Feb 1979 , 1 (1) p91-101 , **ISSN:** 0141-9846--Print **Journal Code:** 7910737
Publishing Model Print
Document type: Journal Article; Research Support, Non-U.S. Gov't
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
In four mature chronically catheterized fetal sheep in utero arterial pressure, heart rate, arterial pH and haematocrit fell during a 16-70% haemorrhage, while Pao2 rose. Plasma vasopressin concentrations increased and were correlated with the percentage of blood volume removed. Following haemorrhage arterial pressure and heart rate were restored within 60 min, while hyperozaemia and acidaemia persisted. Plasma antidiuretic hormone (ADH) concentrations remained the same or increased and were significantly related to the degree of acidaemia. Upon **return** of the **removed blood**, pressure rose

transiently and Pao2 fell; pH remained low and plasma ADH concentrations fell, but were still related to the degree of acidaemia. In three immature, exteriorized **fetuses** (0.4 of term) plasma vasopressin concentrations also rose during haemorrhage. The results indicate that **fetal** plasma vasopressin levels rise during haemorrhage in response both to hypovolaemia and the subsequent acidaemia. Further the response to haemorrhage is present at an early gestational age.
Record Date Created: 19810327
Record Date Completed: 19810327

15/7/56 (Item 56 from file: 155) **Links**
Fulltext available through: STIC Full Text Retrieval Options
MEDLINE(R)

05378825 **PMID:** 17188
Bone marrow transplantation.

van Bekkum D W
Transplantation proceedings **(** UNITED STATES) Mar 1977 , 9 (1) p147-54 ,
ISSN: 0041-1345--Print **Journal Code:** 0243532
Publishing Model Print
Document type: Journal Article; Review
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Improvements in the results of bone marrow transplantation for the treatment of SCID may be expected by employing purified stem-cell concentrates for patients who do not have a compatible sibling available. Refinements in the purification technique and its monitoring are required, however. For the same category of patients it seems worthwhile to continue attempts at restoration with liver cells from fetuses less than 12 weeks of age. In addition, full protection against infections should be provided for patients expected to develop GVHD, and, therefore, such patients should only be treated in centers where reverse isolation and bacteriologic decontamination can be performed. In view of the rarity of the disease, transplanters should agree on a limited number of graft protocols. For the treatment of bone marrow aplasia, attempts to identify the factors that can serve to predict the occurrence of GVHD in compatible host-donor sibling pairs should be continued. Only when the patients who will develop GVHD can be recognized in advance will it be feasible to fully exploit available GVHD reductive measures. In particular the role of the intestinal microflora should be investigated in this respect. Experimental evidence is presented, suggesting an aggravating influence of microflora on GVHD lesions, which are primarily induced by histocompatibility reactions. For such studies with incompatible siblings, the dog is the best available animal model. For the selective isolation of hemopoietic stem cells for transplantation purposes (as one means of reducing GVHD), methods for rapid identification of stem cells and immune competent cells, respectively, have to be developed. In leukemia, more research is necessary on the factors that play a role in the late complications of bone marrow transplantation. The toxicity of aggressive regimens employed in the eradication of the leukemia should be further analyzed. The collection of autologous normal hemopoietic stem cells from leukemic patients as introduced by Dicke et al. warrants further exploration to see whether these **cells** may **replace** the allogeneic transplantation procedure, thus avoiding all the complications generally encountered in GVHD. For all three diseases, it is extremely important to develop a method for the selection of compatible donors among unrelated individuals, because this will at least double the number of

candidates for therapeutic bone marrow transplantation. Current progress in histocompatibility typing in the rhesus monkey and the dog makes these species excellent models for such investigations. (25 Refs.)
Record Date Created: 19770718
Record Date Completed: 19770718

15/7/134 (Item 14 from file: 159) **Links**
Cancerlit

01034515 PMID: 75806065
THYMUS AND BONE MARROW TRANSPLANTATION AND INTESTINAL PROTECTION AS AIDS IN THE THERAPY OF NEOPLASTIC DISEAE: A HYPOTHESIS AND PRELIMINARY EXPERIMENTS.

Ambrus; Ambrus; Stutzman; Klein; Pickren; Webster; Bardon
Dept. Pathol., Roswell Park Mem. Inst. Buffalo, N.Y.
Perspect Cancer Res Treat
1973 ,
165-182 ,
Document Type: JOURNAL ARTICLE
Languages: ENGLISH
Main Citation Owner: NOTNLM
Record type: Completed
The concept is developed that higher than usual doses of chemotherapeutic agents or irradiation might be delivered in advanced neoplastic disease if it were followed by bone marrow or thymus transplantation or if the gastrointestinal tract, in the case of irradiation, were protected. Rhesus monkeys had **bone marrow removed** from the pelvic bones and stored at 4C prior to 800 rads total body irradiation, followed in 24 hr by **reintroduction** of the **marrow**, either into the marrow cavity of the tibia or i.v. Marrow []takes'' were demonstrated by a pronounced leukocytosis in response to five gamma of bacterial lipopolysaccharide. This dose of irradiation resulted in marked leukopenia over 10-15 d in animals not treated with marrow, and subsequent death from hemorrhage and infection. A similar decline in WBC occurred in animals treated with marrow after irradiation, but the trend reversed at about 10 d, and by d. 40, WBC and platelets were near normal; survivors were observed for 2 yr with no appreciable late effects. Challenged with lipopolysaccharide on d. 12 after irradiation, control animals showed very little response while animals protected with homologous bone marrow cells showed some leukocytosis and little leukopenia on d. 12 and a near normal response on d. 18 after irradiation. Similar results were obtained in animals which received lethal doses of chemotherapeutic agents, particularly the alkylating agent AB-103. This agent was tried in pts with advanced bronchogenic carcinoma after **removal** of **marrow cells** to be inj. 24-48 hr after drug treatment. One such pt received 5.6 mg/kg of AB-103, i.v. (the usual therapeutic dose is 0.7 mg/kg/d x 3), followed in 24 hr by autologous bone marrow infusion. He experienced marked objective and subjective improvement. He later received two conventional courses of AB-103 therapy and survived for 15 mo. **Embryonic** thymus transplantation was carried out in 23 Hodgkin's disease pts and in 2 with mycosis fungoides. Approx half of the Hodgkin's disease pts converted to positive skin reactions in one or more tests, and both pts with mycosis fungoides showed improvement in cellular immunity. Nonantigenic thymic humoral factors are currently under study as possible aids in the regeneration of cellular immune competence after intensive therapy. A procedure for gastric intubation with 2-mercaptoethylguanidine prior to high dose total body irradiation of Fischer rats is described. Animals with only the gastrointestinal tract so protected all died after irradiation, as did animals treated only with homologous

marrow infusions, but the combination of treatments resulted in only 1/20 deaths. (33 refs)
Record Date Created: 19761001

29/7/1 (Item 1 from file: 155) **Links**
Fulltext available through: STIC Full Text Retrieval Options
MEDLINE(R)

09650584 **PMID:** 2096034
[Use of the calvarium for bone grafting in cranio-maxillo-facial surgery]

Utilisation de la voute cranienne comme site de greffes osseuses en chirurgie cranio-maxillo-faciale.
Raulo Y; Baruch J
Service de Chirurgie Plastique et Esthetique, Hopital Henri-Mondor, Creteil.
Chirurgie; memoires de l'Academie de chirurgie (FRANCE) 1990 , 116 (4-5) p359-62 , **ISSN:** 0001-4001--Print **Journal Code:** 0236600
Publishing Model Print
Document type: English Abstract; Journal Article
Languages: FRENCH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Bone grafts's traditional donor sites in cranio-maxillo-facial surgery have been for many years and are still in some occasions the ribs, iliac crest and tibia. Bone grafts taken from the calvaria have been used by some surgeons in the past but its wide acceptance was only achieved after Paul Tessier had reported his own experience. The calvaria is composed of inner and outer tables that encloses a layer of cancellous bone called the diploe. A high degree of variability exist with respect to skull thickness. Nevertheless parietal bones is the preferable site for the harvesting of the graft. The **embryonic** origin of the cranium should be responsible for greater survival of the graft. Membranous bone would maintain its volume to a greater extent than endochondral bone when **autografted** in the cranio-facial region. However this remains controversial. Two techniques can be used for the **harvesting** of a calvarial **bone** grafts. A split thickness calvarial graft involves removal of the outer table while leaving the inner layer in place. Its main disadvantage is the relatively thinness of the bone transferred. A full thickness segment of skull involves the cranium cavity be entered. A half of the graft can be split along the diploe space and returned to fill the donor site. The other half is used for reconstruction. It is a more complicated procedure. Cranial grafts have been used in the following cases. Correction of contour defect of the forehead and zygomatic bones, orbital floor reconstruction, restoration of the nasal bridge, bone grafting of the maxilla and mandibule. The advantages are the following: the donor and recipient sites are in adjacent surgical fields, the donor site scar is hidden in the scalp, morbidity associated with removing the graft is almost inexistent. (ABSTRACT TRUNCATED AT 250 WORDS)
Record Date Created: 19910717
Record Date Completed: 19910717

29/7/8 (Item 1 from file: 5) **Links**
Biosis Previews(R)

0001653988 **Biosis No.:** 19664700058089

Development of wool follicles and fibers on autoplastic grafts of stored foetal lamb skin

Book Title: Biology of the skin and hair growth
Author: RUDALL K M; WICKHAM. G A
Author Address: Astbury De. Biophys., Univ. Leeds, Leeds, Engl., UK
p 75-88 1965
Book Publisher: Americal Elsevier Publishing Company, Inc., Canberra, Australia, New York.
Document Type: Book
Record Type: Abstract
Language: Unspecified

Abstract: A technique for studying grafted **fetal** sheep skin was developed. Skin was removed from **fetuses** via hysterotomy, stored at sub-zero temperatures until after the birth of the donor, and then replaced as an **autograft.** Grafts of skin, removed from **fetuses** 69 to 109 days after conception, expanded rapidly. The time of appearance of fibers at the graft surface suggests that excision and storage usually caused a temporary slowing of development of many follicles with a rapid return to their normal rate of development following grafting. The follicle group structure was disorganized in the grafts. Sebaceous glands developed normally but sweat glands were often absent. Ar-rector pili muscles were seldom found in the grafts. Fibers, similar to the sickle fibers of the normal lamb birthcoat, were found in wool **samples removed** from the grafts. This finding suggests that systemic factors peculiar to the **fetus** do not cause the thinning of fibers attributed to a hypothetical pre-natal check which is characteristic of certain early-developing fibers. ABSTRACT AUTHORS: Authors

29/7/9 (Item 2 from file: 5) **Links**

0001430355 **Biosis No.:** 19644500051546
A tumor tissue analogue of the implanting mouse embryo

Author: WILSON IAN B
Author Address: Univ. Coll. N. Wales, Bangor, Wales
Journal: PROC ZOOL SOC LONDON 141 ((1)): p 137-151 1963 1963
Document Type: Article
Record Type: Abstract
Language: Unspecified

Abstract: Transplants of the Harding-Passey melanoma will implant in the mouse uterus only if transferred on days 3-6 of normal pregnancy or pseudopregnancy. Should pregnancy or pseudopregnancy be accompanied by lactation, implantation of melanoma transplants is not delayed (though blastocyst implantation is delayed for about four days) but the period during which the transplants may implant is extended to at least day 8. **Autografts** of body-wall muscle tissue will implant in the uterus within the same period as will melanoma transplants. Tumor **cells injected** in suspensions through the cervix tended to segregate into clumps more or less spaced out and implanted along the uterus. When the transplanted tissues or cell suspensions implanted, they did so invariably in the antimesometrial mucosa, at the site of normal blastocyst implantation. A decidual reaction was elicited only when the transplanted tissues remained free, or relatively so, in the uterine lumen (between days 3-6). If extensive invasion of the uterine mucosa

occurred then there was no decidual reaction even though there was a strong inflammatory reaction to invasion. Decidual tissue "en bloc" is resistant to invasion by melanoma cells. Concurrently with melanoma invasion, pigment granules, from melanoma cell debris, were ingested by epithelial cells immediately adjacent to the invasion sites. ABSTRACT AUTHORS: A. N. Bragg

29/7/11 (Item 4 from file: 5) **Links**
Biosis Previews(R)

0001220795 **Biosis No.**: 19623800018469

[redacted]

Author: COCHRANE ROBERT L; PRASAD M R N; MEYER ROLAND K
Author Address: Dept. Health, Educ., and Welfare, Washington, D. C.
Journal: ENDOCRINOLOGY 70 ((2)): p 228-232 1962 1962
Document Type: Article
Record Type: Abstract
Language: Unspecified

Abstract: A delay in nidation of **embryo** lasting 3 to 18 days was **obtained** by **autografting** the anterior pituitary **gland** to the kidney in rats on the 2nd day of pregnancy. Ovo-implantation was induced in these rats at will; by the daily injection of 1 [mu]g of estrone. Implantation only rarely occurred, and it was followed immediately by death of the **embryos** in rats hypophysectomized on the 2nd day of pregnancy and treated daily with 4 mg of progesterone and 1 [mu]g of estrone starting the 9th day of pregnancy; it never occurred when treatment was not started until the 14th day. The daily use of 1 [mu]g of estrone in lieu of the progesterone-estrone treatment starting on the 9th day or 14th day of pregnancy failed to induce nidation in hypophysectomized untreated rats. A case of implantation of 1 **embryo** previous to estrone treatment and nidation of 8 additional **embryos** after the estrone in a rat bearing a pituitary **autograft** is reported. It is proposed that this phenomenon be called "asynchronous implantation" rather than the misleading terms superfetation and super-implantation. ABSTRACT AUTHORS: Authors

29/7/12 (Item 5 from file: 5) **Links**
Biosis Previews(R)

0000920879 **Biosis No.**: 19583200008440
Factors affecting the survival of the adrenal medulla and associated cortical cells in the anterior chamber of the rabbit's eye

Author: COUPLAND R E
Author Address: U. Leeds, Eng.
Journal: JOUR ENDOCRINOL 15 ((2)): p 162-170 1957 1957
Document Type: Article
Record Type: Abstract
Language: Unspecified

Abstract: A left-sided adrenalectomy was performed and **autografts** were prepared by inserting portions of adrenal medulla and inner zone cortical cells into the anterior chamber of the eye in 84 instances. One implant degenerated. All others were vascularized by the iris and persisted. Implantation was accompanied by a hemorrhagic reaction. The graft was,

however, well vascularized by 72 hours, by which time vessels in the iris had united with those in the graft. The graft included chromaffin cells together with elements of the reticular, juxta-medullary zones and possibly inner zona fasciculata. In a number of animals, in spite of the presence of an intact right adrenal gland, both cortical and medullary cells persisted up to 6 months. Partial or total **removal** of the right adrenal **gland** was followed by proliferation of the cortical elements, but not of chromaffin cells; the proliferating cortical cells assumed a glomerulosa-like pattern at the periphery of the graft. Subcutaneous injections of ACTH (5 mg twice daily) failed to stimulate cellular proliferation in grafts. Homografts of **fetal** and pre-pubertal adrenal glands were examined at times up to 2 months after insertion into the eye. Homograft reactions were not observed. Chromaffin and cortical cells persisted. ABSTRACT AUTHORS: R. E. Coupland

32/7/1 (Item 1 from file: 155) **Links**
Fulltext available through: STIC Full Text Retrieval Options
MEDLINE(R)

09080827 **PMID:** 2670751
Inability of fetal skin to induce allograft tolerance in fetal lambs.

McCullagh P
John Curtin School of Medical Research, Australian National University, Canberra.
Immunology (ENGLAND) Aug 1989 , 67 (4) p489-95 , **ISSN:** 0019-2805--Print **Journal Code:** 0374672
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Fetal lambs of 53-55 days gestation invariably failed to accept skin allografts from **fetal** donors of similar age but retained allografts from adult donors. **Autografts** of skin were accepted by 53-55-day **fetuses**. When the survival of allografts transplanted from fetal donors of a range of gestational ages was examined, skin from fetuses of up to 85 days was rejected but that from a 95-day donor was retained. Histological examination of fetal skin allografts revealed that these were subject to lymphocytic invasion, evident as the entry of lymphatic vessels and extravasation of lymphocytes within the first week after placement. These manifestations of an allograft reaction became more prominent during the following 2 weeks, with graft rejection being evident by the end of a month. Allografts of adult skin were subject to occasional focal lymphocytic infiltration but otherwise healed in uneventfully.
Record Date Created: 19891003
Record Date Completed: 19891003

32/7/4 (Item 4 from file: 155) **Links**
Fulltext available through: STIC Full Text Retrieval Options
MEDLINE(R)

05157102 **PMID:** 821742

[redacted]

Belin R P; Hollingsworth D R; Reid M C; Davis S L; Beihn R
Endocrine research communications (UNITED STATES) 1976 , 3 (2) p133-44

, **ISSN:** 0093-6391--Print **Journal Code:** 0426337
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Our fetal surgical model was utilized to perform in utero **fetal** lamb thyroidectomy and **autograft** transplantation of thyroid tissue to **fetal** thigh at 82-93 days gestation. Successful in utero transplantation was possible in two of six experimental animals. In one twin pregnancy with an unoperated control lamb, observations were continued to age six months. The athyrotic lamb with a thigh autograft was larger at birth and had a transient weak sucking reflex and awkward gait. It then grew and developed normally with no stigmata of cretinism or delay in bone maturation. At age six months an increase in thyroid stimulating hormone (oTSH) was the single distinguishing observation in the twin with the transplant. Although oTSH levels were elevated to age six months, the pituitary continued to be responsive to thyrotropin releasing hormone (TRH) stimulation. These findings suggest that in utero transplantation of thyroid tissue is technically feasible and that the previously described development of in utero cretinism following **fetal** thyroidectomy can be prevented by a functioning **autograft**. This technique will be useful in attempting allograft transplantation in utero.
Record Date Created: 19761029
Record Date Completed: 19761029

32/7/7 (Item 2 from file: 73) **Links**
Fulltext available through: STIC Full Text Retrieval Options

0071386539 **EMBASE No:** 1979118769
Course of development of isolated rat embryonic ectoderm as renal homograft

Levak-Svajger B.; Svajger A.
Dept. Biol., Fac. Med., Univ. Zagreb, Yu-41001 Zagreb, Yugoslavia
Corresp. Author/Affil: : Dept. Biol., Fac. Med., Univ. Zagreb, Yu-41001 Zagreb, Yugoslavia

Experientia (EXPERIENTIA) (Switzerland) June 7, 1979 , 35/2 (258-260)
CODEN: EXPEA **ISSN:** 0014-4754
Document Type: Journal ; Article **Record Type:** Abstract
Language: English

When the isolated head-fold stage rat embryonic ectoderm is grafted under the kidney capsule, it gives rise to a new mesenchyme with the capacity to differentiate into mesodermal tissues.

32/7/10 (Item 5 from file: 73) **Links**
Fulltext available through: STIC Full Text Retrieval Options

0071029935 **EMBASE No:** 1978169730
Growth of locus coeruleus neurons in oculo independent of simultaneously present adrenergic and cholinergic nerves in the iris

Seiger A.; Olson L.

Dept. Histol., Karolinska Inst., Stockholm, Sweden
Corresp. Author/Affil: : Dept. Histol., Karolinska Inst., Stockholm, Sweden

Medical Biology (MED. BIOL.) (United Kingdom) November 29, 1977 , 55/4 (209-223 r7w+caused)
CODEN: MDBYA **ISSN:** 0302-2137
Document Type: Journal **Record Type:** Abstract
Language: English

Fetal brain tissue pieces containing locus coeruleus (LC) neurons were grafted to the anterior eye chamber alone or together with other grafts (irides, sympathetic ganglia or additional LC) in the presence of the sympathetic and parasympathetic part of the autonomic ground plexus of the iris. Specimens were analyzed with quantitative fluorescence microscopy and uptake of [SUP 3H]metaraminol. LC neurons were shown to grow independently of the simultaneous presence of sympathetic fibres in oculo in the following three experimental situations. Fetal LC grafted to normal eyes, analyzed after cessation of the production of the halo of fluorescent fibres on the host irides. Maturated LC neurons in which growth is reinitiated by addition of an iris transplant which becomes completely innervated. Fetal LC neurons placed on or opposite to iris transplants that in turn were introduced into the eye chamber 1 month before. All LC grafts produced halos of fluorescent fibres on both host irides and iris transplants in a restricted zone. Fetal LC grafts produced fluorescent nerve fibres on host irides independent of the removal of the parasympathetic fibres in the irides. Fetal LC grafts were not significantly inhibited in their fibre production on irides where maturated LC grafts had already formed a halo of densely packed fluorescent fibres. When two fetal LC grafts were introduced simultaneously into the same eye chamber both were able to produce fluorescent fibres together on the host iris. Sympathetic ganglion transplants formed normal looking adrenergic plexuses on host irides that were already carrying LC grafts with halos of fluorescent fibres. In conclusion, the fibre production of LC neurons in oculo is independent of the presence of sympathetic, and probably also parasympathetic, and maturated central NA nerves.

32/7/11 (Item 6 from file: 73) **Links**
EMBASE

0070606055 **EMBASE No:** 1976173160

Transformation of the eye stalk into the optic nerve

ZUR UMWANDLUNG DES AUGENSTIELS IN DEN AUGENNERV

Menkes B.; Alexandru C.; Checiu I.
Med. Forsch. Zent., Lab. Embryol., Timisoara, Romania
Corresp. Author/Affil: : Med. Forsch. Zent., Lab. Embryol., Timisoara, Romania

Revue Roumaine de Morphologie, d'Embryologie et de Physiologie - Serie Morphologie et d'Embryologie (REV. ROUM. MORPHOL. EMBRYOL. PHYSIOL. SER. MORPHOL. EMBRYOL.) December 1, 1975 , 21/2 (99-102)
CODEN: RMEMD
Document Type: Journal ; Article **Record Type:** Citation
Language: German

32/7/15 (Item 2 from file: 5) **Links**
Biosis Previews(R)

0000787495 **Biosis No.:** 19542800011239
Skin transplantation in the foetal lamb

Author: SCHINCKEL P G; FERGUSON K A
Journal: AUSTRALIAN JOUR BIOL SCI 6 ((3)): p 533-546 1953 1953
Document Type: Article
Record Type: Abstract
Language: Unspecified

Abstract: Skin **autografts** and homografts were performed in **fetal** lambs between the ages of 80 and 117 days. By means of histological examination following grafting, visual and histological examinations after birth, and by the use of second-set homografts it was established that homografts are actively rejected by the fetus. This rejection gave every indication of belonging to the general class of activity acquired immune responses and in all respects confirmed at the fetal stage the observations of Medewar (1944, 1945) on young adult animals. Homografts in which the ewe was used as donor were also rejected by the fetus, indicating that the reaction was of fetal and not maternal origin. The expts. established that the fetal lamb is capable of making an immune response to the presence of foreign tissue. This finding is at variance with current concepts of the immunological behavior of fetuses. ABSTRACT AUTHORS: Auth. summ

37/7/1 (Item 1 from file: 155) **Links**
Fulltext available through: STIC Full Text Retrieval Options
MEDLINE(R)

07847831 **PMID:** 3455810
Irradiated mandibular autografts update.

Hamaker R C; Singer M I
Archives of otolaryngology--head & neck surgery (UNITED STATES) Mar 1986
, 112 (3) p277-9 , **ISSN:** 0886-4470--Print **Journal Code:** 8603209
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Nineteen patients underwent primary mandibular reconstruction with irradiated mandibular autografts. Ten were successful, with the longest follow-up being 7 1/2 years; nine failed within six months. Although an excellent graft for size and contour, a 53% success rate is no better than any other primary grafting technique. We have abandoned this technique in most situations and presently are evaluating split-rib grafts and intraoperative radiation to in situ mandibles.
Record Date Created: 19860324
Record Date Completed: 19860324

37/7/2 (Item 2 from file: 155) **Links**
Fulltext available through: STIC Full Text Retrieval Options
MEDLINE(R)

03185597 **PMID**: 6066511
Ectopic autografts of blastocysts in mice maintained in delayed implantation.

Kirby D R
Journal of reproduction and fertility (ENGLAND) Dec 1967 , 14 (3) p515-7 , **ISSN**: 0022-4251--Print **Journal Code**: 0376367
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Record Date Created: 19680214
Record Date Completed: 19680214

37/7/5 (Item 2 from file: 73) **Links**
Fulltext available through: STIC Full Text Retrieval Options
EMBASE

0072192366 **EMBASE No**: 1982120984
Transplantation of fetal or neonatal chondrocytes in cartilage defects

TRANSPLANTATION ISOLIERTER CHONDROZYTEN IN GELENKKNORPEL-DEFEKTE
Helbing G.
Klin. Unfallchir. Plast. Wiederherstell. Chir., Univ. Ulm, D-7900 Ulm, Germany
Corresp. Author/Affil: : Klin. Unfallchir. Plast. Wiederherstell. Chir., Univ. Ulm, D-7900 Ulm, Germany

Fortschritte der Medizin (FORTSCHR. MED.) (Germany) July 2, 1982 , 100/3 (83-87)
CODEN: FMDZA **ISSN**: 0015-8178
Document Type: Journal ; Article **Record Type**: Abstract
Language: German **Summary language**: English

Viability and proliferative capacity of the chondrocytes are particularly important for a successful cartilage graft. This can be demonstrated by a new in vitro colony-forming assay. Only fetal or neonatal chondrocytes are considered to be applicable according to these criteria. Single cell suspensions prepared from articular cartilage can be transplanted without technical problems. In animal experiments, articular cartilage regenerated after transplantation of neonatal chondrocytes, whereas only connective tissue was found in controls.

37/7/10 (Item 1 from file: 5) **Links**
Fulltext available through: STIC Full Text Retrieval Options
Biosis Previews(R)

05684029 **Biosis No.**: 197967073024
MAINTENANCE OF PREGNANCY IN THE RAT IN THE ABSENCE OF LUTEINIZING HORMONE

Author: MACDONALD G J (Reprint)
Author Address: DEP ANAT, COLL MED DENT, NJ RUTGERS MED SCH, PISCATAWAY, NJ 08854, USA**USA
Journal: Proceedings of the Society for Experimental Biology and Medicine 159 (3): p 441-443 1978

ISSN: 0037-9727
Document Type: Article
Record Type: Abstract
Language: ENGLISH

Abstract: Pregnant rats were hypophysectomized and pituitary autografted on day 2, the day after sperm were observed in the vaginal lavage. Estradiol-17.beta. (E-17-.beta.) was injected (0.1 .mu.g/day) on days 8-16 to induce implantation and maintain pregnancy. This protocol resulted in a 4 day delay of implantation and day 8 becomes equivalent to day 4 of normal pregnancy. A single dose of LHAS [luteinizing hormone antiserum] (equivalent to 1.4 times the dose necessary to cause abortion on day 8 in the normal pregnant rat) failed to prevent implantation when administered on day 7 or cause fetal resorption when administered on days 11, 12, 13, 14 or 15 (equivalent to days 4 and 7-11). LHAS given on the 2 successive days 13 and 14 (days 9 and 10 equivalent) was also without effect. LHAS apparently causes abortion in the rat by acting on pituitary LH[lutropin]-like material and not on the ovary, developing fetus or placenta.

37/7/11 (Item 2 from file: 5) **Links**
Fulltext available through: STIC Full Text Retrieval Options
Biosis Previews(R)

05063800 **Biosis No.**: 197713089792
IN-VITRO CULTURED SKIN EPITHELIUM AS AUTO GRAFTS FOR BURN PATIENTS

Author: IGEL H J; BOECKMAN C R; KLEIN R J; FREEMAN A E; HERRMAN B J
Journal: American Journal of Pathology 86 (2): p 7A-8A 1977
ISSN: 0002-9440
Document Type: Article
Record Type: Citation
Language: Unspecified

37/7/15 (Item 1 from file: 144) **Links**
Fulltext available through: STIC Full Text Retrieval Options
Pascal

10471152 . PASCAL No.: 92-0674641

Dermal and subdermal tissue filling with **fetal** connective tissue and cartilage, collagen, and silicone : experimental study in the pig compared with clinical results. A new technique of dermis mini-**autograft** injections

HINDERER U T; ESCALONA J
Clin. Mirasierra, Madrid 28034, Spain
Journal: Aesthetic plastic surgery; Aesthetic plastic surgery , 1990, 14 (4) 239-248
ISSN: 0364-216X Availability: INIST-15799; 354000015689640010
No. of Refs.: 47 ref.
Document Type: P (Serial) ; A (Analytic)
Country of Publication: Federal Republic of Germany

blood 1989 73: 1066-1073

In utero gene transfer and expression: a sheep transplantation model

PW Kantoff, AW Flake, MA Eglitis, S Scharf, S Bond, E Gilboa, H Erlich, MR Harrison, ED Zanjani and WF Anderson

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published semimonthly by the American Society of Hematology, 1900 M St, NW, Suite 200, Washington DC 20036.

In Utero Gene Transfer and Expression: A Sheep Transplantation Model

By Philip W. Kantoff, Alan W. Flake, Martin A. Eglitis, Stephen Scharf, Sheldon Bond, Eli Gilboa, Henry Erlich, Michael R. Harrison, Esmail D. Zanjani, and W. French Anderson

Retroviral-mediated gene transfer was used to insert a Neo R gene into fetal sheep hematopoietic cells obtained by exchange transfusion from lambs in utero. After gene transfer the cells were returned to the donor fetus. The lambs were examined after birth for the presence of a functioning Neo R gene. Of ten analyzable animals, six were positive for G418 resistant progenitor cells (CFU-Mix, CFU-C, BFU-E, CFU-E). Two animals were studied for extended periods of time: 8 and 24 months. Each has demonstrated a pattern wherein positive periods are interspersed with times when there were no detectable G418-resistant cells. We conclude that retroviral-mediated gene transfer can be used to insert genes into early progenitor cells of fetal sheep in utero and that the animals can continue to demonstrate blood cells expressing the gene for more than 2 years after birth.

THE INSERTION OF GENES into hematopoietic cells has been greatly facilitated by the use of retroviral vectors.[1-9] Genes can be transferred with high efficiency into mice and, in certain circumstances, can express in the intact animal.[3-5] Two attempts to insert a functioning gene into the hematopoietic system of a large animal have been reported. Our group transferred the human cDNA for adenosine deaminase (ADA) into a number of monkeys using a bone marrow transplantation/retroviral gene transfer protocol.[10] Several animals demonstrated the expression of human ADA in blood cells for several months after transplantation. Stead et al transferred a dihydrofolate reductase (DHFR) gene into several dogs also using a bone marrow transplantation/retroviral gene transfer protocol.[11] They detected methotrexate-resistant colonies (CFU-GM) 3 to 5 weeks posttransplant in one animal. Thus, even though the efficiency is still very low, the feasibility of gene transfer and expression in large animals has been established.

Infants born with ADA deficiency or a large number of other genetic diseases are essentially normal at birth. There are, however, a number of inherited metabolic diseases (eg, Lesch-Nyhan, Tay Sachs) that may produce irreversible damage to the fetus before birth. It is possible that some of these disorders might be treatable if gene therapy could be done safely in utero. To explore the possibility of in utero gene transfer we have developed a sheep transplantation model and have demonstrated that a gene inserted into a fetal lamb's hematopoietic cells is still expressed in the living animal's blood cells over 2 years after transplantation.

MATERIALS AND METHODS

In all cases surgery was performed under general anesthesia (Ketamine: 700 to 1,000 mg; halothane/oxygen). These studies were conducted in fetal sheep at about 93 to 105 days of gestation (normal term, 145 days) over a 2-year period. A total of 20 pregnant Dorset Merino ewes (Ovis aries) with confirmed dates of conception were used in the five experimental groups of three to five animals each. Direct access to fetal circulation was achieved by placing a catheter in the carotid artery of the fetus as described.[12] This catheter was used to both withdraw blood from the fetus and for the infusion of maternal blood or transduced fetal hematopoietic cells. The overall design of the study was to obtain circulating hematopoietic progenitors from the fetus, insert an exogenous gene(s) via retroviral-mediated gene transfer in vitro, and reinfuse the treated cells into the donor fetus (autologous transplantation). The fetuses were then allowed to complete gestation (45 to 62 days later), and the newborn lambs examined for evidence of the exogenous gene at intervals after birth.

Obtaining and processing of fetal blood cells. A total of 30 to 50 mL of blood was obtained from each fetus by exchange transfusion in 5 mL increments using freshly drawn maternal blood for replacement. The heparinized blood was centrifuged at 600 g for 15 minutes and the buffy coat cells separated, washed twice with Iscove's Modified Dulbecco's Medium (IMDM) with 2% fetal calf serum (FCS), and resuspended in IMDM with 10% FCS at 1×10^7 nucleated cells/mL concentration. Approximately 1.5 to 3.5×10^8 mononuclear cells were obtained from each fetus. Although a small portion of these cells were derived from the mother, the number of assayable maternal hematopoietic progenitors in these cell populations were always very low. Furthermore, in repeated attempts we have not been able to grow hematopoietic colonies from blood of adult sheep, while fetal sheep circulating mononuclear cells form colonies in vitro readily. Depending on the logistics of each study, the fetal blood was processed by buffy coat separation within one to 18 hours after withdrawal from the donor animal. Before processing, the blood was kept at room temperature. We have previously determined that storage of fetal sheep blood for periods of up to 48 hours at room temperature does not significantly affect the number or profile of assayable hematopoietic progenitors. In some studies, 1×10^7 nucleated cells from the fetus were set aside as uninfected control cells. However, in most cases all of the isolated buffy coat cells were subjected to the gene transfer procedure.

From the Laboratory of Molecular Hematology, National Heart, Lung, and Blood Institute, National Institutes of Health (NIH), Bethesda, MD; Department of Surgery, University of California, San Francisco; Department of Human Genetics, Cetus Corporation, Emeryville, CA; Memorial Sloan-Kettering Cancer Center, New York; and Veterans Administration Medical Center, University of Nevada School of Medicine, Reno.

Submitted August 5, 1988; accepted November 8, 1988.

Supported in part by Veterans Administration and Grants No. AM2047 and HL40722 from the NIH, and generous support from the G. Harold and Leila Y. Mathers Charitable Foundation, and by March of Dimes Grant No. I-856.

The present address for Dr Kantoff is the Dana Farber Cancer Institute, Harvard Medical School, Boston; the present address for Dr Eglitis is: Genetic Therapy, Inc, Gaithersburg, MD.

Address reprint requests to W. French Anderson, MD, Chief, Laboratory of Molecular Hematology, NHLBI, Bldg 10, Room 7D18, National Institutes of Health, Bethesda, MD 20892.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" *in accordance with 18 U.S.C. section 1734 solely to indicate this fact.*

0006-4971/89/7304-0009$0.00/0

Gene transfer protocol. Two Moloney-based retroviral vectors were used: N2 and SAX. The N2 vector is a Moloney murine leukemia virus-based vector with the viral coding sequences removed and the Neo R gene from the bacterial Tn5 transposon inserted into it as described elsewhere[13,14] and used in previous studies.[4,5,7] The Neo R gene provides protection against the neomycin-like antibiotic G418 (GIBCO, Grand Island, NY). The SAX vector is a derivative of the N2 vector that contains a human ADA cDNA promoted by the SV40 early promoter as previously described.[15] Although the ratio of viral particles to target cells, and the overall length of incubation, varied somewhat in the five experimental series, the following procedure was used in the majority of cases. The virus containing medium (VCM) was diluted 1:3 to 1:10 with IMDM (Biofluids, Rockville, MD), 10% FCS (Hyclone, Logan, VT); polybrene (Aldrich, Milwaukee) was added to a final concentration of 8 μg/mL. The VCM was generated by replacing the supernatant covering a confluent monolayer of virus producing cells with fresh Dulbecco's Minimum Essential Medium (DMEM) (Biofluids) containing 10% FCS (Hyclone). After 20 to 24 hours the VCM was filtered through a 0.22 μm filter (Corning, Wexford, PA) and frozen at −70°C until used. Fetal buffy coat cells were added at a concentration of 2.5×10^5 cells/mL of diluted VCM (10^7 cells in 40 mL; multiplicity of infection: 8:1 viral particles/nucleated cells), mixed thoroughly and incubated for two to five hours. The mixture was then spun at 400 g for 20 minutes at room temperature and the supernatant discarded. The cells were resuspended in an equal volume of freshly prepared diluted VCM/polybrene mixture and incubated overnight (12 to 14 hours) at 37°C (5% CO_2, humidified air). The cells were then pelleted, washed, and resuspended in IMDM, 2% FCS. The cells from each fetus were suspended in 5 to 10 mL IMDM, 2% FCS and reinfused into the respective fetuses; the intravenous (IV) catheter was then removed.

Bone marrow samples and hematopoietic progenitor assays. The presence/activity of the exogenous gene in hematopoietic progenitors was assessed by testing bone marrow cells obtained from newborn lambs for resistance to G418. Aliquots of fetal cells (1 to 6×10^5 cells/mL) after gene transfer, and bone marrow cells (1 to 6×10^5 cells/mL) obtained from the lamb after birth were cultured in plasma clot (CFU-E, BFU-E) or methylcellulose (CFU-Mix, CFU-C, BFU-E) cultures as described.[16,17] Bone marrow samples were aspirated into heparinized syringes from the posterior iliac crest of treated and age-matched control lambs beginning at 1 week after birth and at about monthly intervals thereafter. Buffy coat cells were prepared for culture as well as for DNA and enzyme analyses. The cells were washed twice and resuspended in IMDM, 10% FCS at desired concentrations or pelleted and frozen at −70°C for use in DNA and enzyme analyses. The cells were cultured in the presence or absence of different concentrations of G418 (0.1 to 4 mg/mL), erythropoietin (0.4 IU/mL), and a preparation of PHA-LCM (5% vol/vol) derived from sheep leukocytes. The plates were incubated at 37°C in a humidified atmosphere of 4% CO_2 in air for four (CFU-E) and nine to 12 days (CFU-Mix, CFU-C, BFU-E). Hematopoietic colonies in plasma clot cultures were examined after transfer, fixing, and staining on glass slides,[16] while colonies in methylcellulose cultures were enumerated in situ.[17]

Detection of Neo R sequences in sheep tissue. One microgram of total genomic DNA was subjected to analysis by the technique of polymerase chain reaction (PCR) as described by Saiki et al[18] with the following changes to the reaction constitutents: PCR primers used were 1 μmol/L CP20 and 1 μmol/L CP21, 187.5 μmol/L dCTP, dTTP, dATP, dGTP, and 5 units of *Thermus aquaticus* DNA polymerase. The PCR primer CP20 (CCAGGCTCAAGGCGCGCATGC) and CP21 (TCACGGGTAGCCAACGCTATG) amplify a 170 base pair (bp) fragment from the Neo R gene. The samples were overlayed with 100 μL of mineral oil and subjected to 35 cycles of PCR. The reactions were run in an automated PCR temperature cycling block that allowed annealing of the PCR primers at 55°C and the extension of the annealed primers at 70°C for one minute. After each extension reaction, the samples were heated at 95°C for one minute. After the 35th cycle, the samples were incubated for five minutes at 70°C to allow the amplification products to be fully extended into double-stranded DNA. One-tenth of each reaction (10 μL) was loaded onto a 3% NuSieve, 1% SeaKem agarose gel and electrophoresed in Tris-borate-EDTA. After electrophoresis the gel was denatured and transferred to a Magna Nylon 66 membrane filter (MSI). The oligonucleotide probe (CP22) was phosphorylated with polynucleotide kinase [γ-^{32}P]ATP, purified by spin-dialysis, and had a specific activity of 1.3 μCi/pmol oligomer. CP22 (CCACAGTCGATGAATCCAGAAAAGC) hybridizes to the region of Neo R sequences between the PCR primers. The filter was prehybridized in 5X SSPE, 5X Denhardt's, and 0.5% SDS at 42°C for 15 minutes; 0.1 pmol of CP22 probe was added and hybridized to the filter for 16 hours at 42°C. The filter was washed in 4X SSPE, 0.1% SDS for five minutes at 45°C and autoradiographed for 1.75 hours at −70°C with one intensifying screen (DuPont Cronex Lightning Plus). The positive control DNA, NTSLD1.1, was a gift from Dr Carl Perez and is estimated to contain approximately one copy of the Neo R gene per cell (personal communication). The sheep negative control sample (#40) was prepared as follows: 2 mL whole blood was lysed with the addition of 6 mL 10 mmol/L Tris-Cl, pH 7.5, 10 mmol/L EDTA, 100 mmol/L NaCl, 40 mmol/L dithiothreitol, 200 μg/mL proteinase K and incubated for 16 hours at 55°C. The sample was phenol extracted and ethanol precipitated as described[18] and purified with GeneClean (Bio101). The other DNA samples were prepared as previously described.[10] Neo R-coded phosphotransferase activity in the lysate of bone marrow buffy coat cells was assayed as previously described.[5,19] Briefly, lysates were subjected to electrophoresis on a nondenaturing polyacrylamide gel; the gel was then overlayed with agarose containing kanamycin at 25 μg/mL and 2 nmol/L [γ-^{32}P]ATP (>5,000 Ci/mmol). Subsequently, the gel was blotted with Whatman P81 paper.

RESULTS

Gene transfer was carried out on 20 sheep fetuses: 16 received the N2 vector and four the SAX vector. As with any surgical procedure involving young fetuses there was high perioperative mortality. Eight fetuses were lost (six N2, two SAX); 12 fetuses survived the procedure and were born alive. One of these newborn lambs (no. 4262) died soon after birth and was not examined for evidence of exogenous gene activity. Of the remaining 11 animals, nine had received the N2 vector and two the SAX vectors. One lamb (no. 2517), which received cells transfected with the SAX vector, was lost to study for unrelated reasons (Table 1). The other lamb (no. 2998), which was treated with the SAX vector, exhibited G418 resistant hematopoietic progenitors at the time of death (1 week of age), but was not examined for the presence of a functional ADA gene. Therefore, none of the four fetuses that received the human ADA gene (ie, the SAX vector) were analyzed for the presence of human ADA. Of the ten analyzable animals (nine of whom received the N2 vector and one the SAX vector), six were positive for G418 resistant hematopoietic cells and four were negative 1 week postpartum. The remainder of this report analyzes these data in detail.

Table 1. Experimental Summary

Animal No.	Vector*	Outcome	G418 Resistant Progenitors
2516	SAX	Aborted 1 mo later	—
2517	SAX	Born alive†	—
2518	N2	Died in utero 3 d later	—
2519	N2	Born alive 3/20/86: alive and well‡§	Yes
2997	SAX	Aborted	—
2998	SAX	Born alive, died 1 wk later§	Yes
2999	N2	Born alive, died during transport	Yes
3000	N2	Born alive, died 1 wk later§	Yes
3381	N2	Died in utero 4 d later	—
3382	N2	Born alive, died in transport	No
3383	N2	Mother died 4 d later	—
3384	N2	Died in utero	—
3390	N2	Aborted	—
3391	N2	Born alive, died 2 mo later§	Yes
3392	N2	Born alive 1/13/87: alive and well	No
3900	N2	Born alive 1/13/87: alive and well	No
4218	N2	Born alive 5/11/87: alive and well§	Yes
4261	N2	Aborted	—
4262	N2	Born alive, died 3 d later	—
4263	N2	Born alive 5/23/87: alive and well	No

*Viral particles containing the vector SAX, previously described,[15] were produced by the packaging cell line PA-12.[26] Viral particles containing the vector N2, also previously described,[13,14] were initially produced by PA-12. N2 was later packaged in the cell line PA-317, after Miller et al demonstrated that PA-12 produced helper-contaminated viral supernatants with N2 and N2-based vectors.[27] Animal no. 2519 received virus containing medium (VCM) from PA-12, while no. 4218 received VCM from PA-317. Neither VCM was tested by S^+L^- assay before use, but it is presumed that at least the former was helper-contaminated.

†Newborn was lost to study due to being stolen by an animal rights' group.

‡Still alive and expressing the gene, this ewe was bred to a normal ram and gave birth to a normal lamb about 2 years after transplantation. The newborn (no. 5436) does not exhibit G418-resistant hematopoietic colonies (see Table 7).

§Serum was negative for viremia by S^+L^- assay on PG4 cells[28] under conditions that would detect five or more replication-competent viral particles per milliliter (ie, serum was diluted 1:5 before assay).

In preliminary studies, the effect of different concentrations (0.1, 0.4, 1.2, 1.5, 2, and 4 mg/mL) of G418 on colony formation by normal (uninfected) fetal, newborn, and adult sheep hematopoietic progenitors in vitro was determined. The development of CFU-Mix, CFU-C, BFU-E, and CFU-E derived colonies was inhibited by G418 in a dose dependent fashion (data not shown). Very small numbers of colonies were still detectable at 1.5 mg/mL G418, but were rarely observed at 2 mg/mL G418. In subsequent experiments, therefore, concentrations of 2 mg/mL G418 were used.

Results presented in Table 2 demonstrate the average efficiency of gene transfer into fetal blood hematopoietic progenitors of three animals. In each case an aliquot of fetal buffy coat cells was set aside as uninfected control and cultured along with the infected cells immediately following the completion of the incubation with the VCM. Colony growth was normal in number, size and morphology in the vector-treated group in the absence of G418 selection. At 2 mg/mL of G418, no colonies were observed in cultures of untreated cells (Table 2). By contrast, cultures of the vector-treated cells established with 2 mg/mL G418 exhibited significant numbers of hematopoietic colonies. The G418-resistant colonies were normal in size and morphology and comprised approximately 8% to 17% of the total number of colonies formed in the absence of G418 (Table 2). The percentage of G418-resistant colonies varied among the progenitor classes with CFU-Mix generally exhibiting the highest resistance level. This difference was also noted in some of the newborn lambs and may simply be a reflection of the relatively small numbers of CFU-Mix derived colonies involved and the imprecise nature of their identification.

To assess the presence of a functional Neo R gene in the hematopoietic progenitors of the treated animals, bone marrow cells obtained from the newborn lambs 1 week after birth and at intervals thereafter were assayed for the growth of hematopoietic colonies in the presence or absence of 2 mg/mL G418. With each series of treated animals, bone marrow cells from normal (untreated) age-matched control lambs were also cultured. Results presented in Table 3 demonstrate that a significant number of live birth lambs that were examined (six of ten animals) exhibited detectable levels of G418-resistant colonies at 1 week after birth (about 45 to 62 days after transplantation). In every case, all classes of progenitors assayed were infected (Table 3); bone marrow cells from age-matched control lambs failed to produce significant numbers of colonies in the presence of 2 mg/mL G418 (Table 3, footnote 1). In most studies, cells were cultured at several concentrations to determine whether the percentage of drug resistant colonies might be dependent on the cell concentration used. However, results from one such

Table 2. Efficiency of Gene Transfer into Fetal Lamb Hematopoietic Progenitors In Vitro*

Cells Cultured	Total No. of Colonies/mL			
	CFU-Mix	CFU-C†	BFU-E	CFU-E†
Uninfected				
No G418	104 ± 26	372 ± 42	766 ± 72	1,930 ± 288
2 mg/mL G418	0	0	0	0
% Resistant colonies	0	0	0	0
Infected				
No G418	130 ± 19	393 ± 35	988 ± 68	2,326 ± 304
2 mg/mL G418	23 ± 4	35 ± 5	92 ± 14	342 ± 52
% Resistant colonies	17.7	8.7	9.3	14.7

*Results (mean ± 1 SEM) from animals no. 2519, 3383, and 3391 are presented. Cells were cultured at 4×10^5 cells/mL in the presence or absence of 2 mg/mL G418.

†Data from animals no. 3383 and 3391 only.

Table 3. Effect of G418 on Hematopoietic Colony Formation by Bone Marrow Cells Obtained From Newborn Lambs 1 Week After Birth*

Lamb No.	Total Number of Colonies							
	CFU-Mix		CFU-C		BFU-E		CFU-E	
	−G418	+G418	−G418	+G418	−G418	+G418	−G418	+G418
2519	19	6	94	17	428	171	976	288
2998	52	10	905	75	840	140	—	—
2999	13	3	130	15	107	12	—	—
3000	27	5	260	25	295	25	—	—
3382	34	0	290	0	318	0	1,260	0
3391	51	2	388	18	612	31	2,087	69
3392	176	0	814	0	738	0	1,738	0
3900	47	0	346	0	490	0	1,408	0
4218†	92	9	209	26	280	21	2,104	197
4263	46	0	190	0	582	0	1,986	1

*Values are from 4 × 10^5 cells/mL cultured in the presence or absence of 2 mg/mL G418. Comparable values for age-matched control lambs (n = 7) cultured simultaneously with the experimental samples were (mean ± 1 SEM): CFU-Mix: 87 ± 7 (−G418), 0 (+G418); CFU-C: 264 ± 32 (−G418), 0 (+G418); BFU-E: 479 ± 52 (−G418), 1 (+G418); CFU-E: 2,386 ± 498 (−G418), 5 (+G418).

†Bone marrow cells were obtained five days after birth.

study, presented in Table 4, demonstrate that there was no consistent influence of cell concentration on the percentage of drug resistant colonies recovered.

For a variety of reasons summarized in Table 1, only two of the lambs that showed G418-resistant colonies at 1 week after birth remained available for long-term follow-up studies. One of the losses was due to an accident during transport of the lamb between facilities (Table 1), but the cause of death in the other cases is unknown. Blood obtained at postmortem from three of the animals was examined for evidence of viremia and found to be negative.

As is shown in Table 5, both lambs no. 2519 and 4218 continued to exhibit G418-resistant colonies throughout the study period. Table 5 also demonstrates that lambs found to be negative for the Neo R gene soon after birth continued to be negative up to several months later. Culture results from sheep no. 2519 are shown in more detail in Fig 1. It can be seen that significant fluctuations in the relative incidence of G418-resistant progenitors occurred in this animal; periods of no activity are followed by the reappearance of G418-resistant colonies (Fig 1). These fluctuations did not follow a set pattern. A similar picture was found for animal no. 4218 where at 58 days of age no G418-resistant CFU-Mix, CFU-C, or BFU-E were detected, and only two CFU-E colonies survived the addition of G418, whereas at 124 days the animal was positive again (Table 6).

On several occasions, marrow or peripheral blood mononuclear cells were isolated from lamb no. 2519 and assayed for the presence of neomycin phosphotransferase (NPT) activity, the product of the Neo R gene. Enzyme activity was not detected despite persistent CFU resistance to G418 when the same marrow cells were cultured in vitro. In another animal (no. 3391), treated in a similar manner to no. 2519, NPT activity was detected in marrow cells 6 weeks after birth (13 weeks after transplantation) (data not shown).

DNA was prepared from peripheral blood and marrow mononuclear cells of a number of animals. Digesting the DNA samples with restriction endonucleases followed by Southern blotting and probing with a Neo R gene probe failed to detect vector DNA sequences. However, when DNA samples from animals no. 2519 and 2998 were subjected to sequence amplification by the PCR method,[18] the

Table 4. Effect of Cell Concentration on the In Vitro Expression of G418 Resistance by Bone Marrow Cells Obtained From Lamb No. 2519 at 1 Week After Birth*

Progenitor Cell Assayed	Number of Marrow Cells/mL (× 10^6)	Untreated Age-Matched Control Lamb		Treated Lamb No. 2519	
CFU-Mix	2	0/8	0%	5/13	38%
	4	0/21	0%	6/19	31%
	6	0/20	0%	8/25	32%
Total		0/49	0%	19/57	33%
CFU-C	2	0/84	0%	11/43	25%
	4	0/64	0%	17/94	18%
	6	1/122	0.8%	ND	ND
Total		1/270	0.4%	28/137	20%
BFU-E	2	4/311	1.3%	52/280	18%
	4	0/619	0%	171/428	40%
	6	1/386	0.2%	162/631	26%
Total		5/1,316	0.4%	385/1,339	29%
CFU-E	2	19/806	2.4%	143/451	32%
	4	11/1,880	0.6%	228/976	23%
	6	18/2,864	0.6%	186/1024	18%
Total		48/5,550	0.9%	557/2,456	23%

*Total numbers of colonies counted are shown. The first value is the number of colonies that grew in the presence of G418, the second value is the number formed in the absence of G418. The calculated percentage is then given.

Table 5. Effect of G418 on Hematopoietic Colony Formation by Bone Marrow Cells Obtained From Experimental Lambs at Intervals After Birth

Lamb No.	% G418-Resistant Colonies* (Months after Birth)								
	<1	4	8	12	13	14	15	17	24
2519	28	1.3	0.9	0	8.5	6.4	0	5.3	4.4
3392	0	—	—	0					
3900	0	0	—	0					
4218	6.9	7.8	4.8						
4263	0	—	0						

*Values reflect percentages of total numbers of colonies (CFU-Mix, CFU-C, BFU-E, and CFU-E) that survived the addition of 2 mg/mL G418.

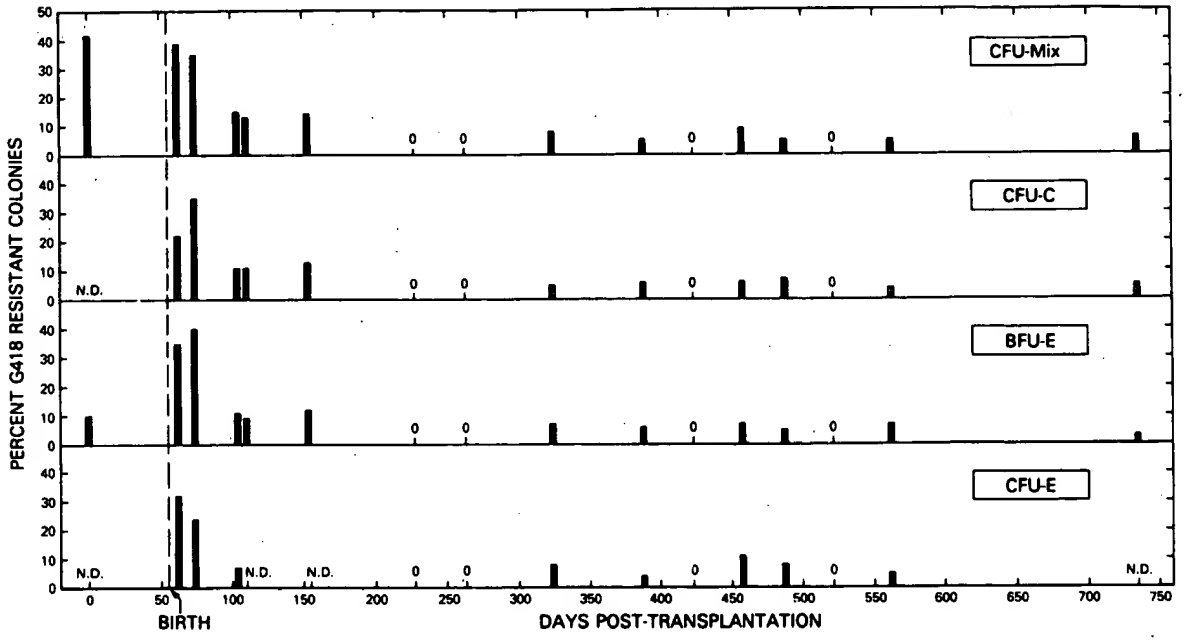

Fig 1. The percent G418 resistant colonies derived from lamb no. 2519 over time. Each value represents mean of results from triplicate cultures established with 2 mg/mL G418. See text for explanation. The dashed line at 55 days represents the day of birth of animal. In all cases values for uninfected age-matched control lambs were <1%.

Neo R sequence was found to be present in blood and bone marrow specimens from animal no. 2519 and in the bone marrow, spleen and thymus samples from animal no. 2998 (Fig 2). Judging from the band intensity of the samples subjected to PCR, the relative proportion of Neo R sequence was estimated to range between 0.1 and 10% for most samples or, on the average, one vector copy per 10 to 1,000 nucleated cells. An occasional sample (eg, Fig 2, lane C) appeared particularly strong, but, since PCR is not a quantitative assay, we doubt that this sample was actually equivalent to the single copy control (Fig 2, lane H).

Animal no. 2519, a female, was bred to a normal (untreated) ram. She was delivered of a healthy lamb at about 2 years of age (about 26 months after transplantation). The data presented in Table 7 show that, despite the presence of significant numbers of G418-resistant progenitors in the mother, no hematopoietic stem cells expressing the Neo R gene were detected in the newborn.

DISCUSSION

The data presented here demonstrate that cloned genes can be transferred into fetal hematopoietic progenitor cells and that expression of the delivered gene can be detected over 2 years after the transplant. The efficiency of gene transfer and expression into hematopoietic progenitors is equal to or higher than that seen in other large animal models in vitro including dog,[20,21] monkey,[10] and human hematopoietic colony assays.[6,7,9] The unique feature about the present study is the persistence of durably transduced stem cells in vivo (particularly since no cytoablation or positive selection in vivo was used), a result not previously seen in any species except the mouse.

Lamb no. 2519, the first positive lamb in our series, has been observed continuously for 2 years posttransplant (Fig 1). Approximately the same percentage of G418-resistant colonies was observed at 75 days posttransplant as observed at 62 days. The number decreased somewhat and stabilized at around 10% to 15% between 104 and 153 days. At days 227 and 263 no G418-resistant colonies were detected (positive controls developed typical G418-resistant colonies). Then at 324 and 388 days a low (4% to 8%), but clearly assayable, level of resistant colonies was demonstrated. G418-resistant colonies were also absent at days 423 and 522 but were detected on days 458 and 488 after transplantation

Table 6. Effect of G418 on Hematopoietic Colony Formation by Bone Marrow Cells Obtained From Animal No. 4218 at Intervals After Birth

Days After Birth	Total Number of Colonies*							
	CFU-Mix		CFU-C		BFU-E		CFU-E	
	−G418	+G418	−G418	+G418	−G418	+G418	−G418	+G418
5	92	9	209	26	280	21	2,104	197
25	48	1	122	4	—	—	947	36
58	56	0	174	0	209	0	1,138	2
124	35	2	79	6	381	39	—	—
247	12	1	61	2	188	5	—	—

*Results from 1 to 6 × 10^5 cells/mL cultured with or without 2 mg/mL G418. No G418 resistant colonies were observed in cultures of bone marrow cells from age-matched control lambs cultured simultaneously with the experimental marrows.

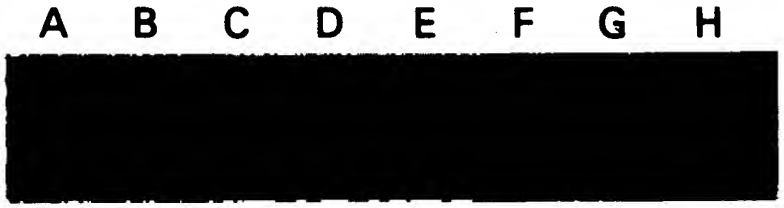

Fig 2. Detection of Neo R sequences in lamb tissue using the polymerase chain reaction method. See text for details. Lanes A, spleen from animal no. 2998 at seven days after birth (DAB); B, thymus from animal no. 2998 seven DAB; C, marrow from animal no. 2998 seven DAB; D, peripheral blood mononuclear cells from animal no. 2519 seven DAB; E, marrow from animal no. 2519 49 DAB; F, marrow from animal no. 2519 seven DAB; G, negative control-sheep blood DNA; H, positive control-NTSLD1.1 DNA.

(Fig 1). This re-emergence of hematopoietic cells that express the Neo R gene, which was also seen in animal no. 4218 (Table 6), is consistent with the hypothesis put forth by Mintz et al[22] and Lemischka et al[23] to explain their data in mice. They postulated that normal hematopoiesis results from the sequential activation of different stem cell clones rather than from an averaged contribution of the entire stem cell pool. Furthermore, Lemischka et al[23] suggested that some stem cells, although replicating to a sufficient extent in vitro to permit proviral integration during the retroviral vector infection process, can return to a quiescent state on transplantation back into the animal and, theoretically, become reactivated to express their exogenous gene later in the lifetime of the animal. The data shown in Fig 1 are consistent with this hypothesis, although it is also possible that our data could be interpreted as simply fluctuations in the total stem cell population.[24]

The physiology of hematopoietic differentiation in the fetal lamb may help to explain why fetal blood cells are susceptible to gene transfer. During the stage of development at which we attempted gene transfer, two important changes are occurring in the hematopoietic system: extensive growth-associated hematopoietic expansion as well as a change in the primary site of hematopoiesis from the liver and spleen to the bone marrow. It is generally believed that the latter process is associated with the seeding of the developing marrow spaces by hematopoietic stem cells delivered via the circulation. It is therefore possible that a large number of early progenitor cells with high self-renewal capacity are in the circulating pool and, therefore, are recoverable by exchange transfusion. These cells (after being infected and reinfused) would represent a small but significant proportion of the overall stem cell pool, thereby allowing the detection of stem cell progeny carrying the vector DNA for many months after transplant. Furthermore, the reinfused cells may have an advantage at "homing" into developing hematopoietic sites in the bone marrow due to their sheer numbers delivered as one large bolus. It is possible, in addition, that the explantation and in vitro treatment of a portion of the total hematopoietic stem cells may confer a proliferative or seeding advantage for these cells, creating an over-representation in the total marrow. We have previously shown that the in utero transplantation of heterologous hematopoietic cells resulted in the creation of long-term chimera in sheep[25] in the absence of cytoablation. Therefore, the reinfused infected cells may compete favorably for unoccupied bone marrow spaces. Nonetheless, we do not understand why the percentages of G418-resistant colonies on days 62 (1 week after birth) and 75 (20 days after birth) are so high.

Table 7. Effect of G418 on Hematopoietic Colony Formation by Bone Marrow Cells Obtained From Sheep No. 2519 at 2 Years After Birth and From Her Offspring at 7 Days After Birth

	Total Number of Colonies*					
	CFU-Mix		CFU-C		BFU-E	
Animal (#)	−G418	+G418	−G418	+G418	−G418	+G418
Control (3958)	20	0	82	0	408	0
Mother (2519)	18	1	44	2	480	14
Offspring (5463)	28	0	125	0	564	0

*Results from 6 × 10^5 cells/mL cultured with or without 2 mg/mL G418.

Successful long-term engraftment of stem cells after in vitro gene transfer has been achieved in the mouse model but not in primate[10] or dog models[11] in which cytoablation is used to afford a selective survival and proliferative advantage to infected stem cells. The apparent increased efficiency of gene transfer in this sheep model does not appear to be explained primarily by species differences in susceptibility to virus infection (or vector gene expression) but rather to an inherent difference between progenitor cells derived from the fetus *v* the adult. We have infected the bone marrow cells of an adult sheep with the N2 virus and transplanted these cells into a heterologous recipient fetal lamb. Hematopoietic chimerism in the newborn lamb was achieved as measured by hemoglobin markers, but no evidence of Neo R gene activity was detected as measured by G418-resistant colony growth. Thus, our in utero protocol was unsuccessful when adult marrow cells were used instead of fetal cells.

Furthermore, preliminary experiments from our laboratory evaluating the efficiency of infection of human fetal cord blood (obtained at the time of normal, but premature, deliveries) has demonstrated that 20- to 24-week fetal cells can be as much as tenfold more efficiently infected than newborn blood or adult bone marrow. Therefore, the present evidence suggests that fetal hematopoietic progenitor cells (at least in the sheep and human) are inherently more susceptible to retroviral gene transfer (with subsequent expression of the exogenous gene) than newborn or adult blood cells.

The demonstration of vector DNA sequences in the blood and/or bone marrow of two animals (no. 2519 and 2998), and NPT activity in the bone marrow of another animal (no. 3391), confirm that gene transfer and expression has occurred. However, there is a clear quantitative discrepancy between the high frequency of in vitro G418-resistant colo-

nies and the low abundance of vector DNA sequence in whole marrow and blood as evidenced by Southern blotting and PCR analysis. At the level of G418 used for selection, only negligible levels of background drug-resistant colonies were detected in progenitors grown from uninfected marrow, indicating that the behavior of the infected marrow was truly distinct.

The apparent discrepancy between the PCR data, where 0.1% to 10% of the marrow cells contain vector sequence, and the significantly higher percentages of G418-resistant colonies obtained from in vitro culture of the same bone marrow could be a manifestation of different populations of cells being analyzed. As has been demonstrated in the murine model,[22-24] only a small proportion of stem cells at any one time appear to be differentiating into more mature cells in vivo. Although a large proportion of the progenitor pool is not contributing substantially to the overall pool of mature cells (which is the population analyzed in DNA studies), when the progenitor cells from the animal are placed in culture under the influence of growth factors, most of the progenitor cells might then be capable of differentiating and thereby possibly "unmasking" dormant vector-transduced cells. Such behavior has not been reported in the in vivo murine or other animal models, so that this may be a unique characteristic of the sheep system. Alternatively, ovine hematopoietic progenitors may be sensitive to a "cross-protection" phenomenon, where successfully transduced cells protect otherwise G418-sensitive nontransduced progenitors, yielding a spuriously high proportion of G418-resistant colonies. However, we have not yet detected such putative "satellite" colonies in human, canine, or murine studies either in liquid culture or in semi-solid medium. These issues are currently under investigation.

In summary, we report here successful gene transfer into fetal hematopoietic cells in vivo using a retroviral vector as well as the demonstration in a large animal of long-term expression in vivo after in utero gene transfer. Although we did not analyze lymphoid cells, the 2-year persistence of myeloid/erythroid G418-resistant cells suggests that pluripotent stem cells were infected. This study opens the possibility for studying genes that participate in or modualte the hematopoietic process during development and extends to the fetus the potential application of a gene therapy approach for genetic diseases, particularly for those disorders that might require prenatal intervention for optimal results. Except for those diseases like ADA deficiency where there should be a positive selection pressure for the growth of treated cells,[1] most genetic diseases will probably require cytoablation of the patient's own marrow, with all the risks that this procedure involves. If it can be substantiated that gene transfer via fetal cell autologous transplantation obviates the need for cytoablation, then gene therapy in utero may be a reasonable therapeutic option in some cases.

ACKNOWLEDGMENT

The authors would like to thank Sherrie Beck and Lysa Sahr for their excellent technical assistance.

REFERENCES

1. Anderson WF: Prospects for human gene therapy. Science 226:401, 1984

2. Williams DA, Lemischka IR, Nathan DG, Mulligan RC: Introduction of new genetic material into pluripotent haematopoietic stem cells of the mouse. Nature 310:476, 1984

3. Dick JE, Magli MC, Huszar D, Phillips RA, Bernstein A: Introduction of a selectable gene into primitive stem cells capable of long-term reconstitution of the hematopoietic system of W/Wv mice. Cell 42:71, 1985

4. Keller G, Paige E, Gilboa E, Wagner EF: Expression of a foreign gene in myeloid and lymphoid cells derived from multipotent haematopoietic precursors. Nature 318:149, 1985

5. Eglitis ME, Kantoff PW, Gilboa E, Anderson WF: Gene expression in mice after high efficiency retroviral-mediated gene transfer. Science 230:1395, 1985

6. Gruber HE, Finley KD, Hershberg RM, Katzman SS, Laikind PK, Seegmiller JE, Friedmann T, Yee JK, Jolly DJ: Retroviral vector-mediated gene transfer into human hematopoietic progenitor cells. Science 230:1057, 1985

7. Hock RA, Miller AD: Retrovirus-mediated transfer and expression of drug resistance genes in human hematopoietic progenitor cells. Nature 320:275, 1986

8. Belmont JW, Henkel-Tigges J, Chang SM, Wager-Smith K, Kellems RE, Dick JE, Magli MC, Phillips RA, Bernstein A, Caskey CT: Expression of human adenosine deaminase in murine haematopoietic progenitor cells following retroviral transfer. Nature 322:385, 1986

9. Hogge DE, Humphries RK: Gene transfer to primary normal and malignant human hemopoietic progenitors using recombinant retroviruses. Blood 69:611, 1987

10. Kantoff PW, Gillio A, McLachlin J, Bordignon C, Eglitis MA, Kernan NA, Moen RC, Kohn DB, Yu S-F, Karson E, Karlsson S, Zwiebel JA, Gilboa E, Blaese RM, Nienhuis AW, O'Reilly RJ, Anderson WF: Expression of human adenosine deaminase in nonhuman primates after retroviral mediated gene transfer. J Exp Med 166:219, 1987

11. Stead B, Kwok WW, Storb R, Miller DA: Canine model for gene therapy: Inefficient gene expression in dogs reconstituted with autologous marrow infected with retroviral vectors. Blood 71:742, 1988

12. Harrison MR, Jester JA, Ross NA: Correction of congenital diaphragmatic hernia in utero. I. The model: Intrathoracic balloon produces fatal pulmonary hypoplasia. Surgery 88:174, 1980

13. Armentano D, Yu S-F, Kantoff PW, von Ruden T, Anderson WF, Gilboa E: Effect of internal viral sequences on the utility of retroviral vectors. J Virol 61:1647, 1987

14. Gilboa E, Eglitis MA, Kantoff PW, Anderson WF: Transfer and expression of cloned genes using retroviral vectors. BioTechniques 4:504, 1986

15. Kantoff PW, Kohn DB, Mitsuya H, Armentano D, Sieberg M, Zwiebel JA, Eglitis MA, McLachlin J, Wiginton DA, Hutton JJ, Horowitz SD, Gilboa E, Blaese RM, Anderson WF: Correction of adenosine deaminase deficiency in cultured human T and B cells by retrovirus-mediated gene transfer. Proc Natl Acad Sci USA 83:6563, 1986

16. Roodman GD, Zanjani ED: Endogenous erythroid colony forming cells in the fetal and newborn sheep. J Lab Clin Med 94:699, 1979

17. Ash RC, Detrick RA, Zanjani ED: Studies of human pluripotential hematopoietic stem cells (CFU-GEMM) in vitro. Blood 58:309, 1981

18. Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich

HA, Arnheim N: Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 230:1350, 1985

19. Reiss B, Sprengel R, Will H, Schaller H: A new sensitive method for qualitative and quantitative assay of neomycin phosphotransferase in crude cell extracts. Gene 30:211, 1984

20. Kwok WW, Schuening F, Stead RB, Miller AS: Retroviral transfer of genes into canine hemopoietic cells in culture: A model for human gene therapy. Proc Natl Acad Sci USA 83:4552, 1986

21. Eglitis MA, Kantoff PW, Jolly JD, Jones JB, Anderson WF, Lothrop CD Jr: Gene transfer into hematopoietic progenitor cells from normal and cyclic hematopoietic dogs using retroviral vectors. Blood 71:717, 1988

22. Mintz B, Anthony D, Litwin S: Monoclonal derivation of mouse myeloid and lymphoid lineages from totipotent hematopoietic stem cells experimentally engrafted in fetal hosts. Proc Natl Acad Sci USA 81:7835, 1984

23. Lemischka IR, Raulet DH, Mulligan RC: Developmental potential and dynamic behavior of hematopoietic stem cells. Cell 45:917, 1986

24. Snodgrass R, Keller G: Clonal fluctuation within the haematopoietic system of mice reconstituted with retrovirus-infected stem cells. EMBO J 6:3955, 1987

25. Flake AW, Harrison MR, Adzick NC, Zanjani ED: Transplantation of fetal hematopoietic cells in utero; the creation of hematopoietic chimeras. Sciencre 233:776, 1986

26. Miller AD, Law MF, Verma IM: Generation of helper-free amphotropic retroviruses that transduce a dominant-acting methotrexate-resistant dihydrofolate reductase gene. Mol Cell Biol 5:431, 1985

27. Miller AD, Buttimore C: Redesign of retrovirus packaging cell lines to avoid recombination leading to helper virus production. Mol Cell Biol 6:2895, 1986

28. Haapala DK, Robey WG, Oroszlan SD, Tsai WP: Isolation from cats of an endogenous type C virus with a novel envelope glycoprotein. J Virol 53:827, 1985